Dual RNA regulatory control of a Staphylococcus aureus virulence factor.

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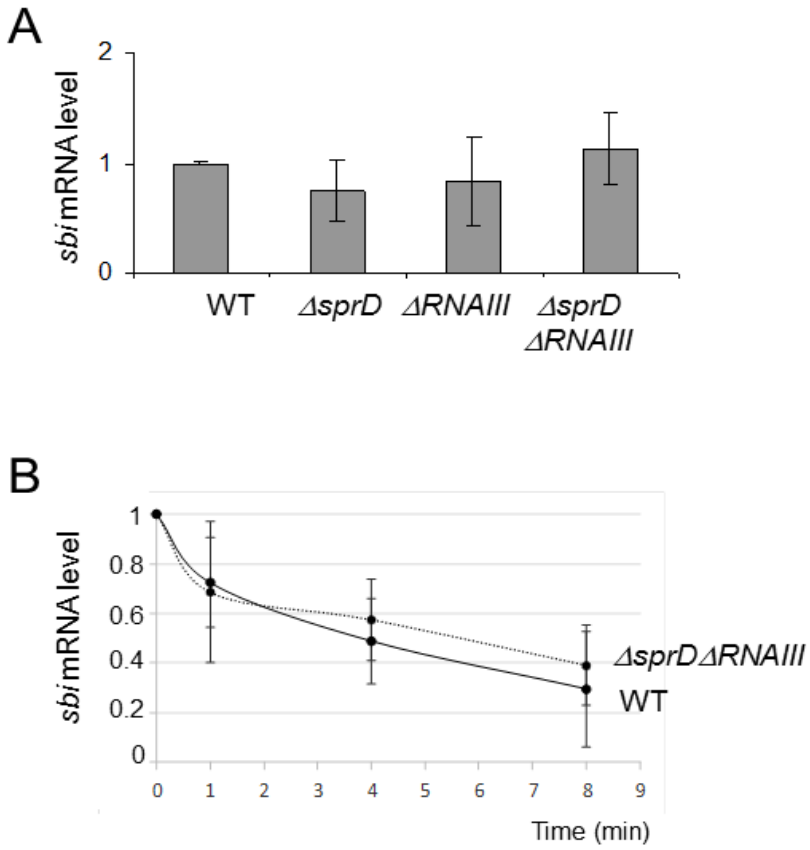
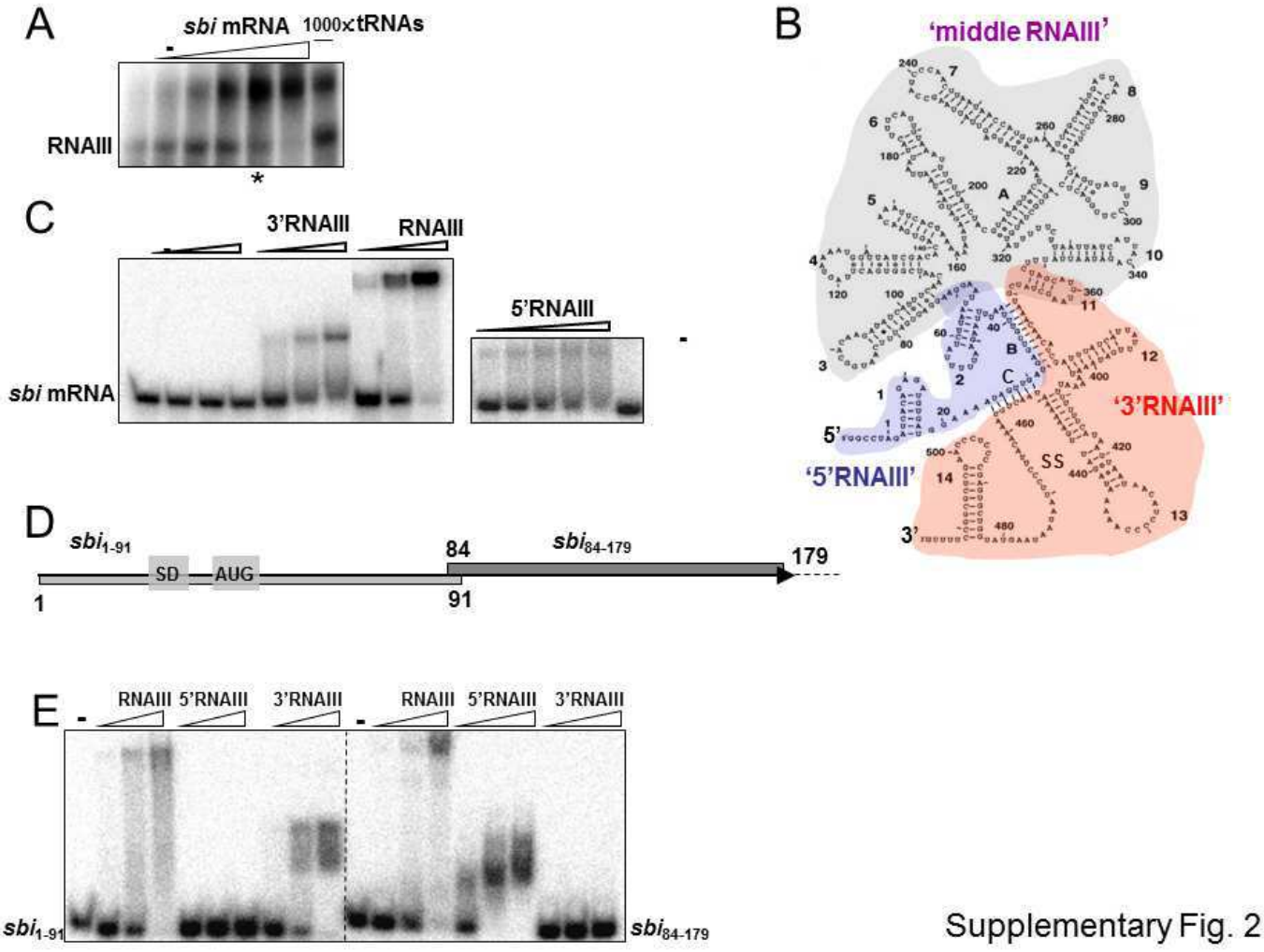
Supplementary data



Supplementary Figure 1. **RNAIII and SprD RNAs do not control Sbi expression at the RNA level.** (A) Quantification by PCR of the *sbi* mRNA levels at the E phase (OD_{600nm}: 3) in wild-type (wt) and in Δ*sprD*, Δ*rnaIII* and Δ*sprD*-Δ*rnaIII S. aureus* HG001 isogenic deletion strains. Both, SprD and RNAIII, are expressed at this growth point (Figures 1B and 1D). The *sbi* mRNA expression levels of the three mutants were calculated relative to the value measured for the wt strain. The error bars indicate the mean values derived from three independent experiments. For the quantitative real-time PCR (qRT–PCR), cDNAs were prepared using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT–PCR experiments were performed using RealMasterMix SYBR ROX (5 PRIME) with the primers listed in Supplementary Table 2. Three independent experiments were performed, with independent RNA purifications. The *hu* and *ssrA* genes were used for normalization. (B) Evaluation of the half-life of *sbi* mRNA in the strains HG001 (WT) and HG001Δ*sprD*Δ*rnaIII* (Δ*sprD*Δ*rnaIII*).

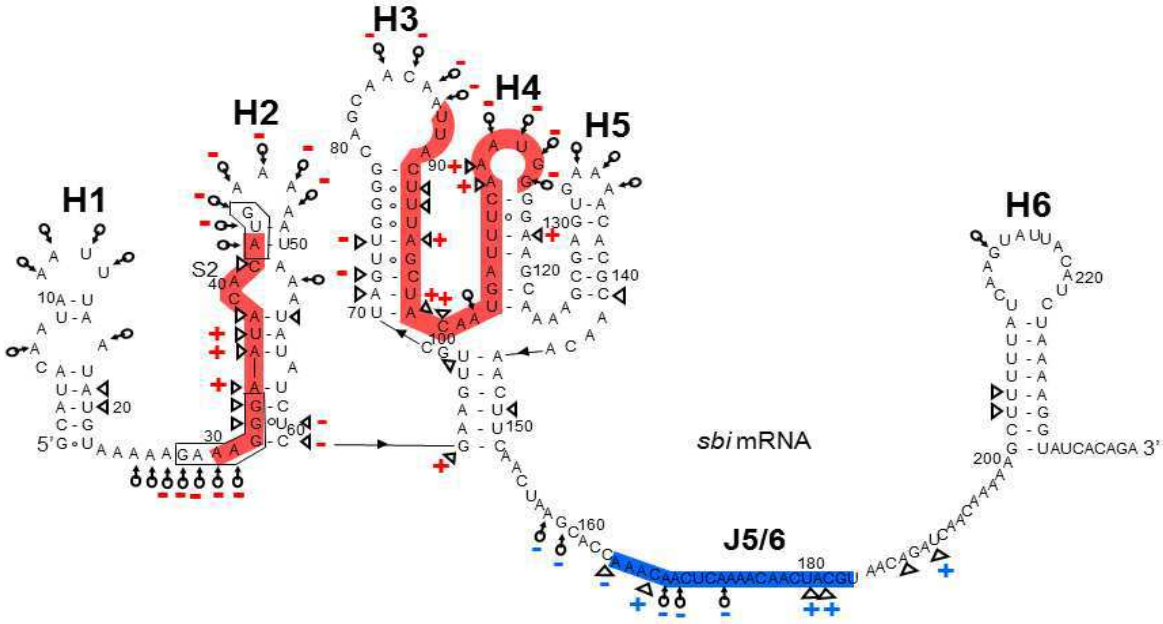
sbi mRNA half-life was measured in the presence of rifampicin (200 mg/ml) at mid-exponential growth phase (after 4h of growth). qRT-PCR conditions were as for panel (A). The half-life was given as the time where 50% of the RNA was degraded. Four independent experiments were performed, with independent RNA purifications.



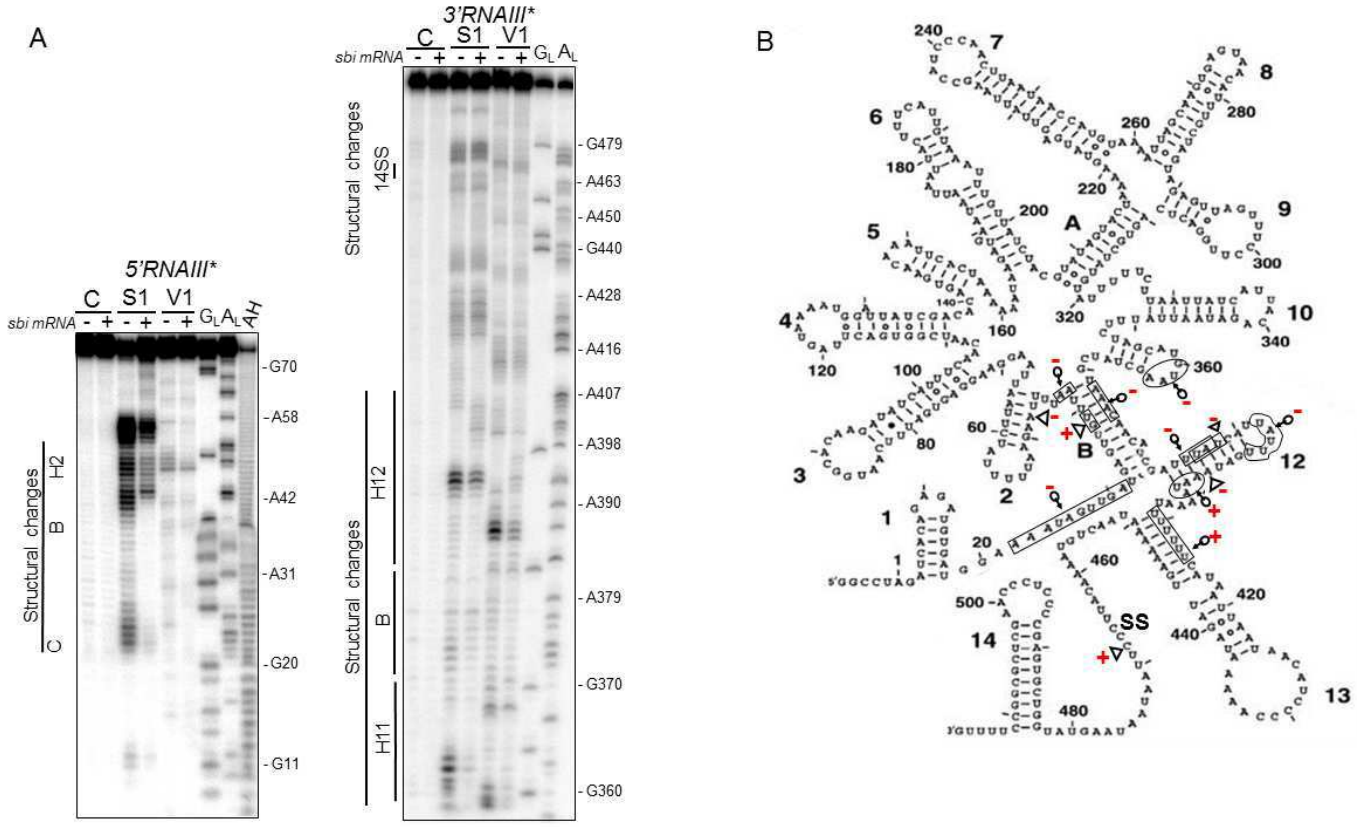
Supplementary Fig. 2

Supplementary Figure 2. **RNAIII 5' and 3' domains interact at distinct sites on *sbi* mRNA.** (**A**) Native gel retardation assays of labeled RNAIII with increasing amounts of unlabeled $sbi_{1\text{-}179}$ mRNA (0.1, 0.2, 0.5, 1 and 2 µM)**.** The asterisk indicates the *sbi* mRNA/RNAIII molar ratio used to perform the competition assays with a 1000-fold molar excess of total yeast tRNAs. (**B**) Deletion mutants constructed from and presented on the RNAIII secondary structure. 'middle RNAIII' is gray and corresponds to stem-loops 3-11 (grey), 5'RNAIII is blue and contains stem-loops 1-2 with half B and half C, and 3'RNAIII is red and encloses stem-loops 11-14. (**C**) Both its 5'- and 3'-domains are involved in RNAIII's interaction with *sbi* mRNA. Native gel retardation assays were used to show complex formation between labeled *sbi* mRNA and: full-length RNAIII (RNAIII; 0.1, 0.5 and 2 µM,

left to right); 3'-domain (3'RNAIII; 0.1, 0.5 and 2 µM,); central domain (Middle RNAIII; 0.1, 0.5 and 2 µM,); or 5'-domain (5'RNAIII; 0.1, 0.2, 0.5, 1 and 2.5 µM). (**D**) Schematic representation and location of the $sbi_{1\text{-}91}$ and $sbi_{84\text{-}179}$ constructs on *sbi* mRNA. Only $sbi_{1\text{-}91}$ contains the translation initiation signals. (**E**) RNAIII 3'-domain binds at the 5'-end of the *sbi* mRNA, while the 5'-domain of RNAIII interacts with the 3'-end of the *sbi* mRNA fragment. Native gel retardation assays were used to show complex formation between purified labeled 1-91 nts *sbi* mRNA ($sbi_{1\text{-}91*}$) or 84-179 nts *sbi* mRNA ($sbi_{84\text{-}179*}$) constructs with increasing amounts (0.2, 1.0 and 2.5 µM) of unlabeled full-length RNAIII, 5'-RNAIII or 3'-RNAIII.

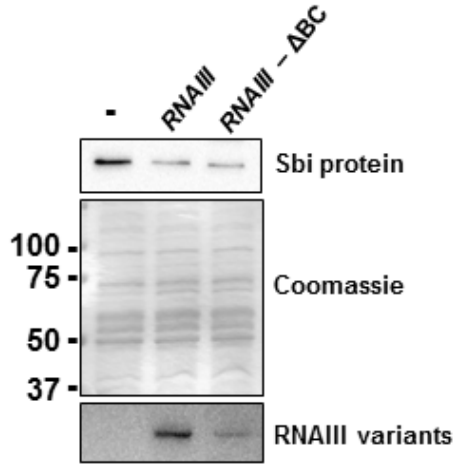


Supplementary Figure 3. The **5'-secondary structure of *sbi* mRNA.** Secondary structure model of the *sbi* mRNA 5'-end (nts 1-238) of *S. aureus* strain N315. Solution probing data provided experimental support for the proposed structure. A conformation of only 62 nucleotides from the *sbi* mRNA 5'-end was already reported (Chabelskaya *et al*, 2010). Since the RNAIII-*sbi* mRNA interactions are extensive and complicated, the probing data were collected for a longer *sbi* mRNA fragment. Triangles indicate V1 cuts; arrows capped by circles indicate S1 cuts. Shown are the structural changes induced by *sbi* mRNA binding of 5'RNAIII (blue), 3'RNAIII (red), and full-length RNAIII. The boxed nucleotides are the *sbi* mRNA TIS.

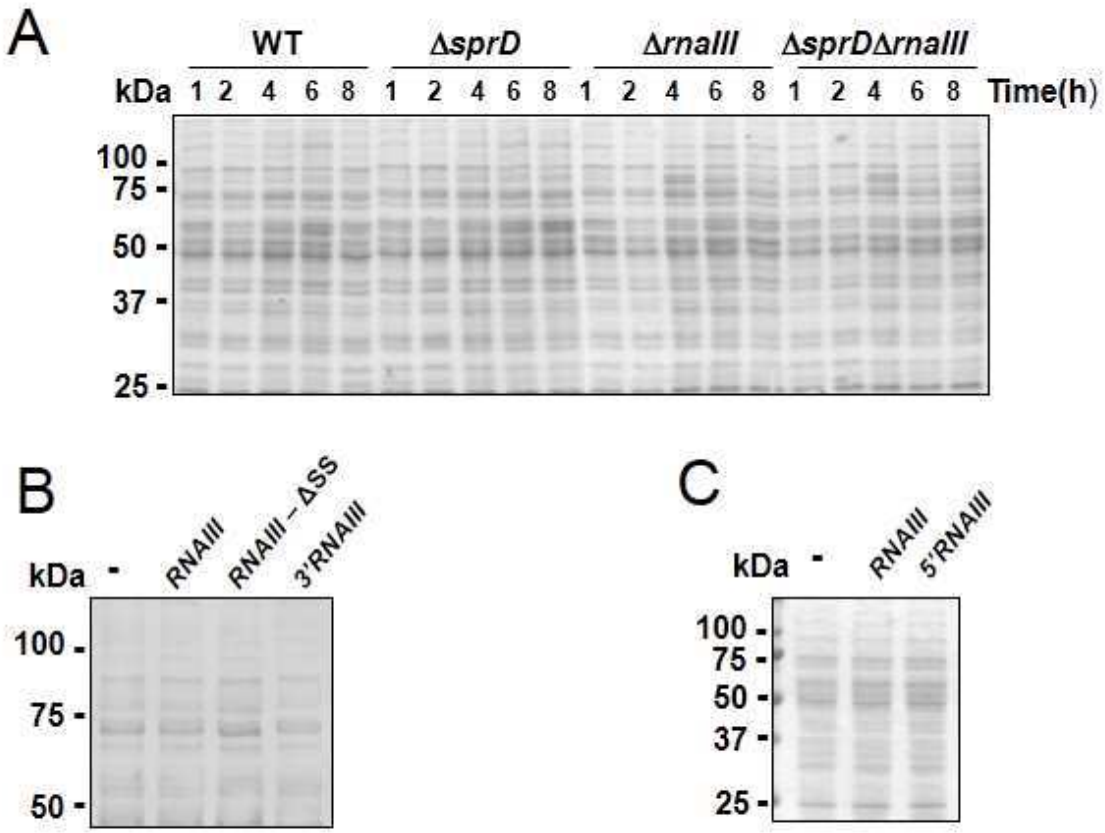


Supplementary Figure 4. **Structural analysis of the conformational changes within the structure of RNAIII when in complex with *sbi* mRNA.** (**A**) Conformational changes induced by the *sbi* mRNA on 5'RNAIII (left) or 3'RNAIII (right), detected by structural probes in solution. Shown are autoradiograms of the cleavage products of 5'-labeled RNAIII fragments by Nuclease S1 and RNase V1 in the presence (+) or absence (–) of *sbi* mRNA. Track C, incubation controls; track G_L, RNase T_1 hydrolysis ladders; track A_L, RNase U_2 hydrolysis ladders; track AH, alkaline hydrolysis ladders. (**B**) The RNAIII secondary structure (Benito *et al.* 2000) supported by the probing data presented in this report, showing the structural changes induced by complex formation with *sbi mRNA*. Triangles indicate V1 cuts; arrows capped by circles indicate S1 cuts. The structural changes induced by *sbi* mRNA binding with RNAIII are red, and the outlined nucleotides are those that are subjected to reactivity changes. Complex formation between *sbi* mRNA and 5'RNAIII induced structural changes located 22-44 nucleotides from the RNAIII 5'-end (part of stems B and C and H2). Binding between

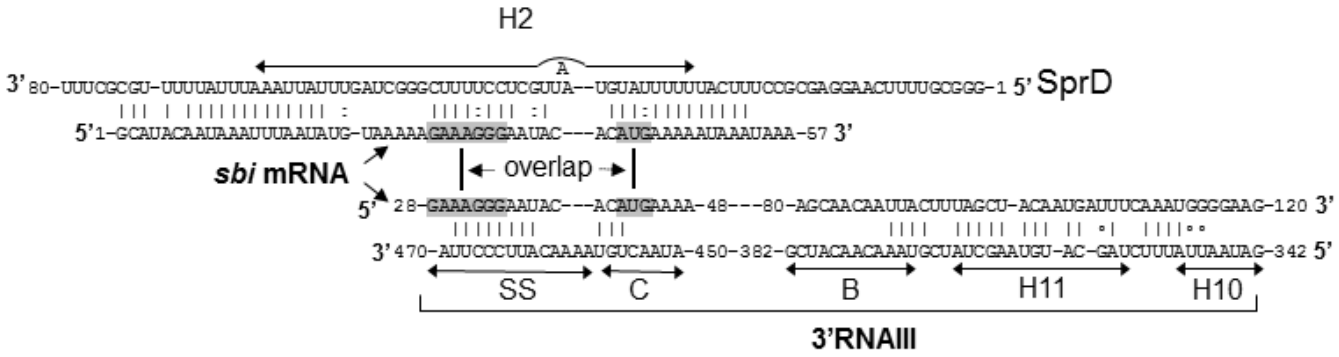
the *sbi* mRNA and 3'RNAIII induced structural changes located at loop 11, stem-loop 12, part of stem 13, and at SS.



Supplementary Figure 5. A *RNAIII-ΔBC* mutant is still able for governance of Sbi protein expression *in vivo*. The expression of protein Sbi at the exponential phase of growth in strain HG001*ΔrnaIII* complemented with pCN38Ω*RNAIII* (RNAIII) or pCN38Ω*RNAIII-ΔBC* (RNAIII-ΔBC). Total protein amounts loaded and stained by Coomassie are shown. The lower panel depicts the Northern blot expression levels of RNAIII and mutant.



Supplementary Figure 6. Internal protein controls. Coomassie staining of the samples presented in Figures 1A (**A**), 6B (**B**) and 6C (**C**).



Supplementary Figure 7. **Proposed pairings between the *sbi* mRNA translation initiation signals, SprD, and RNAIII**. Pairings are based on the experimental data provided in this report and from our previous work (18). The structural domains of the two RNAs are indicated and the gray highlighting corresponds to the *sbi* mRNA TIS.

Supplementary Table 1. Strains used in and constructed for this study

E. coli		**References**
DH5α	F$^-$ ϕ80 Δ*lacZ* Δ*M15* Δ(*lacZA-argF*)*U169 deoR recA1 endA1 hsdR17* (r_K^- m_K^-) *phoA supE44* λ^- *thi-1 gyrA96 relA1*	3
S. aureus		
RN4220	Restriction-defective derivative of 8325-4	4
HG001	*rsbU* restored strain 8325, lysogenic for phages ϕ11, ϕ12, and ϕ13	5
HG001 Δ*rnaIIIa*	HG001 deleted for *rnaIII* by homologous recombination	2
HG001 Δ*rnaIII*	HG001 deleted for *rnaIII;* rnaIII::cat86	This work
HG001 Δ*sprD*	HG001 deleted for *sprD*	This work
HG001 Δ*sprD*Δ*rnaIII*	HG001 deleted for *sprD* and *rnaIII;* rnaIII::cat86	This work

Supplementary Table 2. DNA primers used in this study.

DNAs	**Sequences**	**Purposes**
Anti1640	GGCGCTCCTTGAAAACGCCC	SprD Northern
NB3'RNAIII	TCAAATAATGATAAATCGATGTTGT	RNAIII Northern
T7RNAIIIfor	TAATACGACTCACTATAGGGCCTAGATCACAGAGATGT	RNAIII transcription
T7RNAIIIrev	CAAAAGGCCGCGAGCTTGGGA	
SBIforTR	TAATACGACTCACTATAGGCATACAATAAATTTAATATGTAA	*sbi* mRNA transcription, toeprint
SBIrevTR179	GTTGTTTTGAGTTGTTTGGTGCT	
SbiT7rev238	TCTGTGATACCTTTTAGATGTA	
T7rev_Sbi_5'90	GTAATTGTTGCTGCCCCAACT	$sbi_{1\text{-}91}$ transcription
SBIT7delta84	TAATACGACTCACTATAGGCAATTACTTTAGCTACAAT	$sbi_{84\text{-}179}$ transcription
RNAIII1_2rev	CTTCCTTAATTAAGATAAAAATTC	5'RNAIII transcription
RNAIII11_14for	TAATACGACTCACTATAGGGCTAGCATGTAAGCTATCGTAAACAACA	3'RNAIII transcription
RNAIII2_10for	TAATACGACTCACTATAGGGGTAATTAAGGAAGGAGTGATTTCA	'middle' RNAIII transcription
RNAIII3_11rev	GATAGCTTACATGCTAGAAATAAT	
Revdelta14	CAGTTATTTTTTCAATCTATTT	RNAIII-ΔSS&14 mutation, for *in vitro* transcription
D14_mut_tb14rev	CAAAAGGCCGCGAGCCTTTCCTTTGCTCACGACCATACTTATTA	RNAIII-lutL14 mutation, for *in vitro* transcription
D14ssfor	ATAGATTGAAAAAATAACTGTAAAAAATAATAAGTATGGTCGTGA	RNAIII-ΔSS mutation, for *in vitro* transcription and *in vivo* expression
D14ssrev	TCACGACCATACTTATTATTTTTTACAGTTATTTTTTCAATCTAT	
RNAIIIdeltaBC	TAATACGACTCACTATAGGGCCTAGATCACAGAGATGTGATGGTTAAGAATTTTTATCTTAATT	'*RNAIII-ΔBC*' RNAIII transcription
rnaIIIDelta_11_12_for	AATTATCATTACAGATAATTATAAACAACATCTTTTTTTCATAATTAATAACA	'*RNAIII-Δ11,12*' RNAIII transcription
rnaIIIDelta_11_12_rev	TGTTATTAATTATGAAAAAAAGATGTTGTTTATAATTATCTGTAATGATAATT	
RNAIII3EcoRI	AGTAGGAATTCCAACGCGAAAATATACCTGTAT	RNAIII plasmid

RNAIII5PstI	AGTTCTGCAGATACGTGGCAAACTGGTCAAT	
new3'11_12_13_14_for	AGCATGTTTTAATATAACTAGTTATCATTACAGATAATTATT	*in vivo* 3'RNAIII expression
new3'11_12_13_14_rev	AATAATTATCTGTAATGATAACTAGTTATATTAAAACATGCT	

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